

What is claimed is:

1. A method for the analysis of a target sequence in a sample,

5 said method comprising:

a. contacting the sample with at least one pair of probes (Probe A and Probe B), wherein:

i) Probe A is comprised of a nucleotide sequence, which hybridizes

to a target region of both wanted and unwanted DNA or RNA and is labeled with a first fluorophore at the end which, upon hybridization is closest to Probe B; and

10 ii) Probe B is comprised of a nucleotide sequence which hybridizes to the target region of unwanted DNA or RNA adjacent to the target region of Probe A and is labeled with a quencher at the end which, upon hybridization is closest to Probe A.

b. detecting, identifying or quantitating the hybridization of Probe A to the target sequence, under suitable hybridization conditions, wherein the presence or amount of wanted DNA or

15 RNA present in the sample can be positively correlated with the fluorescence of the fluorophore of Probe A.

2. The method of claim 2, where the method is performed by fluorescence in situ hybridization.

20 3. The method of claim 1, wherein Probe A and Probe B are high affinity probes.

4. The method of claims 1, wherein Probe A and Probe B are peptide nucleic acid (PNA) probes.

25 5. The method of claim 1, wherein one of more of the probes has a probing nucleobase sequence of 11-16 subunits in length.

6. The method of claim 1, wherein Probe A comprises the following nucleotide sequence:
GCT-TCT-CGT-CCG-TTC.

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7. The method of claim 1 or 6, wherein Probe B comprises the following nucleotide sequence: ACT-TCA-AAG-GAG-CAA.

5 8. The method of claim 1, wherein Probe A consists essentially of the following nucleotide sequence: GCT-TCT-CGT-CCG-TTC and the fluorophore.

9. The method of claim 1 or 6, wherein Probe B consists essentially of the following nucleotide sequence: ACT-TCA-AAG-GAG-CAA and the quencher.

10 10. The method of claim 1, wherein Probe A has the following nucleotide sequence: GCT-TCT-CGT-CCG-TTC.

11. The method of claim 1, wherein Probe B has the following nucleotide sequence: ACT-TCA-AAG-GAG-CAA.

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12. The method of claim 1 where Probe A is labeled with the fluorophore at the probe terminus closest to the binding site of Probe B, and Probe B is labeled with a quencher at the probe terminus closest to the binding site of Probe A.

20 13. The method of claim 1 or 13 where Probes A and B are labeled internally.

14. The method of claim 1, wherein Probe B is further labeled with a fluorophore at the opposite end and wherein which fluorophore has a different emission spectrum than the fluorophore on Probe A.

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15. The method of claim 1, wherein, upon hybridization, the two PNA probes are separated by a distance of between from about one to about five nucleotide bases.

16. The method of claim 1, wherein the target sequence is obtained from a cell or tissue.

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17. The method of claim 16, wherein the cell or tissue has been manipulated to preserve the target sequence therein.

18. The method of claim 17, wherein the manipulation includes fixation, freezing or
5 desiccation.

19. The method of claim 1, wherein step (b) of the method detects, identifies, or quantitates the presence or amount of at least one species of a microorganism in the sample.

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20. The method of claim 19 wherein the target sequence was isolated from a microorganism exposed to at least one antimicrobial agent and the presence of amount of wanted DNA or RNA is taken to be indicative of an effect of the antimicrobial agent on the microorganism.

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21. The method of claim 1, wherein the detection, identification or quantitation step is indicative of a condition of medical interest.

22. A kit suitable for performing an assay which detects the presence, absence or amount
20 of target sequence in a sample, wherein said kit comprises a Probe A comprised of a nucleotide sequence, which hybridizes to a target region of both wanted and unwanted DNA or RNA and is labeled with a fluorophore at the end which, upon hybridization is closest to the adjacent target region for Probe B; and a Probe B comprised of a nucleotide sequence which hybridizes to the target region of unwanted DNA or RNA adjacent to the
25 target region of Probe A and is labeled with a quencher at the end which, upon hybridization is closest to Probe A.

23. The kit of claim 22, wherein Probe A comprises the following nucleotide sequence: GCT-TCT-CGT-CCG-TTC and is labeled with the fluorophore.

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24. The kit of claim 22 or 23, wherein Probe B comprises the following nucleotide sequence: ACT-TCA-AAG-GAG-CAA and is labeled with the quencher.

25. The kit of claim 22, wherein Probe A consists essentially of the following nucleotide sequence: GCT-TCT-CGT-CCG-TTC and is labeled with a fluorophore.

26. The kit of claim 22 or 23, wherein Probe B consists essentially of the following nucleotide sequence: ACT-TCA-AAG-GAG-CAA and is labeled with the quencher.

27. The kit of claim 22, wherein Probe A has the following nucleotide sequence: GCT-TCT-CGT-CCG-TTC and is labeled with the fluorophore.

28. The kit of claim 22 or 27, wherein Probe B has the following nucleotide sequence: ACT-TCA-AAG-GAG-CAA and is labeled with the quencher.

29. The kit of claim 22, wherein Probe A has the following nucleotide sequence: GCT-TCT-CGT-CCG-TTC and is labeled with a fluorophore; and Probe B has the following nucleotide sequence: ACT-TCA-AAG-GAG-CAA and is labeled with the quencher.

30. The kit of claim 22, wherein the kit is adapted for use in a fluorescence in-situ hybridization assay.

31. The kit of claim 22, wherein the kit is adapted to detect organisms in food, beverages, water, pharmaceutical products, personal care products, dairy products or environmental samples.

32. The kit of claim 22, wherein the kit is adapted to test raw materials, products or processes.

33. The kit of claim 22, wherein the kit is adapted to examine clinical samples.

34. The kit of claim 33, wherein the clinical samples are clinical specimens or equipment, fixtures and products used to treat humans or animals.

5 35. The kit of claim 22, wherein the kit is adapted to detect a target sequence which is specific for a genetically based disease or is specific for a predisposition to a genetically based disease.

10 36. The kit of claims 22, wherein the kit is adapted to detect a target sequence associated with a disease selected from the group consisting of 5-Thalassemia, sickle cell anemia, Factor-V Leiden, cystic fibrosis and cancer related targets such as p53, pLO, BRC-1 and BRC-2.

15 37. The kit of claim 22, wherein the kit is adapted to detect a target sequence in a forensic technique.

38. The kit of claim 37, wherein the forensic technique is at least one of prenatal screening, paternity testing, identity confirmation or crime investigation.